

## CLAIMS

1. A method for determining whether a test compound inhibits RNA synthesis of a positive strand RNA virus, comprising:

contacting an isolated replicase complex for the positive strand RNA virus, an isolated viral replicon template RNA for the positive strand RNA virus, a labeled nucleotide analog, and the test compound, under conditions sufficient for *in vitro* RNA synthesis, to form a newly synthesized RNA population comprising the labeled nucleotide analog;

detecting the newly synthesized RNA population comprising the labeled nucleotide analog;

quantitating the newly synthesized RNA population comprising the labeled nucleotide analog to provide a test RNA amount; and

comparing the test RNA amount with a control RNA amount of a control newly synthesized RNA population comprising the labeled nucleotide analog produced in the absence of the test compound, wherein a decrease in the test RNA amount compared to the control RNA amount indicates that the test compound inhibits RNA synthesis of the positive strand RNA virus.

2. The method of Claim 1, wherein contacting further comprises contacting with 2'-O-methyl-5-methyluridine-5'- triphosphate.

3. The method of Claim 1, further comprising providing the isolated replicase complex and the isolated viral replicon template RNA by:

transfecting a cell line with a viral replicon RNA or a DNA template for a viral replicon to provide a transfected cell line,

incubating the transfected cell line under conditions suitable for production of viral replicase complexes, and

isolating the replicase complexes and the viral replicon template RNA from the cell membrane fraction of the transfected cells.

4. The method of Claim 3, wherein the positive strand RNA virus is Hepatitis C Virus and the DNA template for a viral replicon is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 4, or SEQ ID NO: 5.

5. The method of Claim 1, further comprising providing the isolated replicase complex comprising the viral replicon template RNA by  
incubating a positive strand RNA virus infected primary cell or cell line under conditions suitable for production of viral replicase complexes, and  
isolating the replicase complexes comprising the viral replicon template RNA from the cell membrane fraction of the infected cells.

6. The method of Claim 1, wherein the positive strand RNA virus is Hepatitis C Virus.

7. The method of Claim 1, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody, an analog which can be recognized via a high specificity binding reaction, or an analog directly detectable as a result of a physical property of the analog.

8. The method of Claim 7, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody.

9. The method of Claim 8, wherein the labeled nucleotide analog is Br-UTP.

10. The method of Claim 8, wherein detecting comprises contacting the newly synthesized RNA with an antibody specific for the labeled nucleotide analog and immuno-precipitating the newly synthesized RNA comprising the labeled nucleotide analog to form immuno-precipitated RNA.

11. The method of Claim 10, wherein the labeled nucleotide analog is Br-UTP and the specific antibody is an anti-BrdU antibody.

12. The method of Claim 10, wherein quantitating the newly synthesized RNA comprises performing real time PCR on the immuno-precipitated RNA.
13. The method of Claim 1, wherein the test compound is an RNA elongation inhibitor.
14. The method of Claim 1, wherein the test compound is an RNA synthesis initiation inhibitor.
15. The method of Claim 1, wherein the test compound is a replicase complex activity inhibitor.
16. The method of Claim 7, wherein the analog directly detectable as a result of a physical property of the analog is a radioactive nucleotide.
17. The method of Claim 16, wherein detecting comprises gel electrophoresis.

18. A method for quantitating newly initiated RNA of a positive strand RNA virus comprising:

contacting an isolated replicase complex for the positive strand RNA virus, an isolated viral replicon template RNA for the positive strand RNA virus, and a labeled nucleotide analog, under conditions sufficient for *in vitro* RNA synthesis, to form a newly synthesized RNA population comprising the labeled nucleotide analog;

hybridizing a probe and the newly synthesized RNA population comprising the labeled nucleotide analog, under stringent hybridization conditions, wherein the probe is complementary to at least a portion of a transcription initiation region of the newly synthesized RNA population;

digesting unhybridized, single-stranded RNA with a single-strand specific ribonuclease to form a protected RNA population comprising the labeled nucleotide analog;

detecting the protected RNA population comprising the labeled nucleotide analog;  
and

quantitating the protected RNA population comprising the labeled nucleotide analog.

19. The method of Claim 18, wherein contacting further comprises contacting with 2'-O-methyl-5-methyluridine-5'- triphosphate.

20. The method of Claim 18, further comprising providing the isolated replicase complex and the isolated viral replicon template RNA by:

transfecting a human hepatosoma cell line with a viral replicon RNA or a DNA template for a viral replicon to provide a transfected cell line,

incubating the transfected cell line under conditions suitable for production of viral replicase complexes, and

isolating the replicase complexes comprising the viral replicon template RNA from the cell membrane fraction of the transfected cells.

21. The method of Claim 20, wherein the DNA template for the viral replicon is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 4, or SEQ ID NO: 5.

22. The method of Claim 18, further comprising providing the isolated replicase complex comprising the viral replicon template RNA by:

incubating a positive strand RNA virus infected primary cell or cell line under conditions suitable for production of viral replicase complexes, and

isolating the replicase complexes comprising the viral replicon template RNA from the cell membrane fraction of the infected cells.

23. The method of Claim 18, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody, an analog which can be recognized via a high specificity binding reaction, or an analog directly detectable as a result of a physical property of the analog.

24. The method of Claim 23, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody.

25. The method of Claim 24, wherein the labeled nucleotide analog is Br-UTP.

26. The method of Claim 24, wherein detecting comprises contacting the newly synthesized RNA with an antibody specific for the labeled nucleotide analog and immuno-precipitating the newly synthesized RNA comprising the labeled nucleotide analog to form immuno-precipitated RNA.

27. The method of Claim 26, wherein the labeled nucleotide analog is Br-UTP and the specific antibody is an anti-BrdU antibody.

28. The method of Claim 27, wherein quantitating the newly synthesized RNA comprises performing real time PCR on the immuno-precipitated RNA.

29. The method of Claim 23, wherein the analog directly detectable as a result of a physical property of the analog is a radioactive nucleotide.

30. The method of Claim 29, wherein detecting comprises gel electrophoresis.

31. A method for determining whether a test compound is an RNA synthesis initiation inhibitor of a positive strand RNA virus comprising:

contacting an isolated replicase complex for the positive strand RNA virus, an isolated viral replicon template RNA for the positive strand RNA virus, a labeled nucleotide analog, and the test compound, under conditions sufficient for *in vitro* RNA synthesis, to form a newly synthesized RNA population comprising the labeled nucleotide analog;

hybridizing a probe and the newly synthesized RNA population comprising the labeled nucleotide analog, under stringent hybridization conditions, wherein the probe is complementary to at least a portion of an initiation region of the newly synthesized RNA population;

digesting unhybridized, single-stranded RNA with a single-strand specific ribonuclease to form a protected RNA population comprising the labeled nucleotide analog;

detecting the protected RNA population comprising the labeled nucleotide analog;

quantitating the protected RNA population comprising the labeled nucleotide analog to provide a test RNA amount; and

comparing the test RNA amount with a control RNA amount of protected RNA comprising the labeled nucleotide analog but produced in the absence of the test compound, wherein a decrease in the test RNA amount compared to the control RNA amount indicates that the test compound inhibits RNA synthesis initiation of the positive strand RNA virus.

32. The method of Claim 31, wherein contacting further comprises contacting with a 2'-O-methyl-5-methyluridine-5'- triphosphate.

33. The method of Claim 31, further comprising providing the isolated replicase complex and the isolated viral replicon template RNA by:

transfecting a human hepatosoma cell line with a viral replicon RNA or a DNA template for a viral replicon to provide a transfected cell line,

incubating the transfected cell line under conditions suitable for production of viral replicase complexes, and

isolating the replicase complexes comprising the viral replicon template RNA from the cell membrane fraction of the transfected cells.

34. The method of Claim 33, wherein the DNA template for a viral replicon is SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 4, or SEQ ID NO: 5.

35. The method of Claim 31, further comprising providing the isolated replicase complex comprising the viral replicon template RNA by:

incubating a positive strand RNA virus infected primary cell or cell line under conditions suitable for production of viral replicase complexes, and

isolating the replicase complexes comprising the viral replicon template RNA from the cell membrane fraction of the infected cells.

36. The method of Claim 31, wherein the positive strand RNA virus is Hepatitis C Virus.

37. The method of Claim 31, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody, an analog which can be recognized via a high specificity binding reaction, or an analog directly detectable as a result of a physical property of the analog.

38. The method of Claim 37, wherein the labeled nucleotide analog is an analog capable of being recognized by a specific antibody.

39. The method of Claim 37, wherein the labeled nucleotide analog is Br-UTP.

40. The method of Claim 37, wherein detecting comprises contacting the newly synthesized RNA with an antibody specific for the labeled nucleotide analog and immuno-precipitating the newly synthesized RNA comprising the labeled nucleotide analog to form immuno-precipitated RNA.

41. The method of Claim 40, wherein the labeled nucleotide analog is Br-UTP and the specific antibody is an anti-BrdU antibody.

42. The method of Claim 41, wherein quantitating the newly synthesized RNA comprises performing real time PCR on the immuno-precipitated RNA.

43. The method of Claim 37, wherein the analog directly detectable as a result of a physical property of the analog is a radioactive nucleotide.

44. The method of Claim 43, wherein detecting comprises gel electrophoresis.

45. A kit for screening a test compound for inhibition of RNA synthesis of a positive strand RNA virus, comprising:

an isolated replicase complex for the positive strand RNA virus;

an isolated viral replicon template RNA for the positive strand RNA virus;

instructions for use; and

a buffer and nucleoside triphosphates sufficient for production of newly synthesized viral replicon RNA.

46. The kit of Claim 45, further comprising a labeled nucleotide analog.

47. The kit of Claim 46, further comprising a means for detecting the labeled nucleotide analog.

48. The kit of Claim 45, further comprising a means for quantitating the labeled nucleotide analog.

49. The kit of Claim 45, further comprising a primer, a single-strand specific ribonuclease, and instructions for performing a ribonuclease protection assay.

50. The kit of Claim 45, wherein the nucleoside triphosphates comprise 2'-O-methyl-5-methyluridine-5'- triphosphate.